IN THE SPECIFICATION:

Please amend the specification as follows:

On page 5, delete the fourth full paragraph, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

"FLGK:" As used herein, "FLGK" refers to a mutant of human FLGK which is characterized by the amino acid sequence of SEQ ID NO:2. As compared to human FLGK, FLGK contains the following amino acid substitutions: Cys-488 → Ala, Cys-584 → Ser, Leu-457 → Val, and has an additional five amino acid residues at the N-terminus (residues 1-5 of SEQ ID NO:2) (Ser-Ala-Ala-Gly-Thr).

On page 11, delete the last paragraph beginning on page 11 and ending on page 12, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

FIG. 3 provides a structure-based sequence alignment of human fibroblast growth factor receptor 1 (FGFR1) (SEQ ID NO:6), human fibroblast growth factor receptor 2 (FGFR2) (SEQ ID NO:7), human fibroblast growth factor receptor 3 (FGFR3) (SEQ ID NO:8), human fibroblast growth factor receptor 4 (FGFR4) (SEQ ID NO:9), a *D. malanogaster* homolog (DFGFR1) (SEQ ID NO:10), a *C. elegans* homolog (EGL-15) (SEQ ID NO:11) and insulin receptor tyrosine kinase (IRK) (SEQ ID NO:12).

On page 12, delete the third full paragraph, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

FIGS. 6A (SEQ ID NOS. 13-30, respectively, in order of appearance) and 6B (SEQ ID NOS 31-41, respectively, in order of appearance) are amino acid sequence alignments of the catalytic domains of PTKs, including receptor and non-receptor type PTKs. FIG. 6A

depicts one representative member from each of the eighteen subfamilies of receptor tyrosine kinases. FIG. 6B depicts one representative member from each of the subfamilies of cytoplasmic tyrosine kinases. In FIGS. 6A and 6B highly conserved residues are boxed. The position of the glycine-rich domain, kinase insert, catalytic loop, and activation loop are indicated. The numbering is for human FGF-receptor.

On page 24, delete the last paragraph beginning on page 24 and ending on page 25, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

A structure-based sequence alignment of the tyrosine kinase domains of human fibroblast growth factor receptor 1 (human FLGK; labelled FGFR1), human fibroblast growth factor receptors 2, 3 and 4 (labelled FGFR2, FGFR3 and FGFR4, respectively), *a D. melanogaster* homologue (labelled DFDFR1), a *C elegans* homologue (labelled EGL-15) and insulin receptor kinase (labelled IRK), is shown in FIG. 3. The sequence of FLGK, which is not shown in FIG. 3 is identical to the sequence of FGFR1 except that FLGK has the following amino acid substitutions and additions: Cys-488 → Ala, Cys-584 → Ser, Leu-457 → Val and an additional five N-terminal amino acids (residues 1-5 of SEQ ID NO:2) (Ser-Ala-Ala-Gly-Thr). The secondary structure assignments for FGFR1 and IRK were obtained using the Kabsch and Sander algorithm (Kabsch and Sander, 1983) as implemented in PROCHECK (Laskowski *et al.*, 1993). In the FGF receptor sequences, a period represents sequence identity to FGFR1. In the IRK sequence, residues that are identical to FGFR1 are highlighted. A hyphen denotes an insertion.

On page 27, delete the first full paragraph, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

The catalytic loop of protein kinases lies between secondary structure elements αE and β7 and contains an invariant aspartic acid residue (Asp-623 in FLGK) which serves as the

catalytic base in the phosphotransfer reaction, abstracting the proton from the hydroxyl group of the substrate tyrosine, serine or threonine. The catalytic loop sequence of FLGK comprises at least residues His-621 to Asn- 628 (amino acid sequence residues 166-173 of SEQ ID NO:1 HRDLAARN), and is identical to that for IRK and most receptor and non-receptor PTKs.

On page 30, delete the last paragraph beginning on page 30 and ending on page 31, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

A recombinant baculovirus (Pharmingen, CA) was engineered to encode the protein of SEQ ID NO:3. Compared to the sequence of human FLGK (SEQ ID NO:1), the protein of SEQ ID NO:3 has a cleavable histidine tag (MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPSSR) fused to the N-terminus to aid in protein purification. Also, three amino acid substitutions were introduced: Cys-488→Ala, Cys-584→Ser and Leu-457→Val. The two cysteine substitutions were made to prevent the formation of disulfide-linked oligomers, which occurs for the native protein. The substitution Leu-457→Val was necessary to introduce a NcoI cloning site near Met-456. The codon for Tyr-766 (TAC) was changed to a stop codon (TAG) and a HindIII-cloning site was generated following this stop codon. These substitutions were introduced into the full length cDNA of human FLGK (SEQ ID NO:4) in m13MP19 by site-directed mutagenesis using an in vitro mutagenesis kit according to the manufacturer's protocol (Amersham). The resulting construct was digested with NcoI and HindIII and was ligated into appropriately digested pBlueBac HistagB (Invitrogen). Transfection of insect cells (Sf9) was performed with the BaculoGold transfection system according to the manufacturer's protocol (Pharmingen). Following identification of positive plaques, the recombinant baculovirus was amplified to high titer (5 x 10^7 virus particles/ml). Sf9 cells were grown in 175-cm^2 flasks to a density of 2-3x10^7 per flask and infected with recombinant baculovirus with a multiplicity of infection (MOI) of ten (10). After 48 hrs., cells were harvested by centrifugation at 3,000g for 35 min. at 4°C and then lysed in lysis buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol,

1.5 mM $MgCl_2$, 1% Triton X-100, 10 μg/ml aprotonin, 10 μg/ml leupeptin and 1 mM phenylmethylsulfonyl fluoride (PMSF)). Lysates were centrifuged in a Sorval RC 5C centrifuge (Dupont) for 1 hr at 4°C at 40,000g followed by ultracentrifugation in an XL-80 ultracentrifuge (Beckman) at 100,000g for 1 hr. After centrifugation, the clarified lysate was passed over a Ni^{2+}-chelating column (Pharmacia), and the bound histidine-tagged fusion protein was eluted with 100 mM imidazole (pH 7.5). Pooled fractions were loaded onto a Mono Q anion exchange column (Pharmacia) and eluted with a NaCl gradient from 0 to 500 mM. The fractions containing the fusion protein were concentrated in a Centricon-30 (Amicon), and the histidine tag was removed by overnight digestion with enterokinase (Biozyme) at 20°C. The digestion was terminated by the addition of aprotonin, leupeptin, PMSF, TPCK (tosyl-L-phenylalanine chloromethyl ketone) and bovine pancreatic trypsin inhibitor (BPTI). The cleaved kinase domain was then separated from the histidine tag on a Superose 12 size-exclusion column (Pharmacia). The eluted kinase domain was further purified on a Mono Q column. The purified kinase domain was analyzed by N-terminal sequencing and mass spectrometry. Five amino acids (SAAGT) (residues 1-5 of SEQ ID NO:2) remained from the histidine tag. The predicted molecular mass was confirmed by mass spectrometry. The amino acid sequence of the purified protein (FLGK) is provided in SEQ ID NO:2.

CONCLUSION

As the above-presented amendments and remarks address and overcome all of the rejections presented by the Examiner, withdrawal of the rejections and allowance of the claims are respectfully requested.

If the Examiner has any questions concerning this application, he or she is requested to contact the undersigned.

Respectfully submitted,

Date July 23, 2001 By _____

FOLEY & LARDNER
Washington Harbour
3000 K Street, N.W., Suite 500
Washington, D.C. 20007-5109
Telephone: (202) 672-5475
Facsimile: (202) 672-5399

Beth A. Burrous
Attorney for Applicant
Registration No. 35,087

Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicant(s) hereby petition for any needed extension of time.

MARKED UP VERSION SHOWING CHANGES MADE:

IN THE SPECIFICATION:

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the glycine-rich domain, kinase insert, catalytic loop, and activation loop are indicated. The numbering is for human FGF-receptor.

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The catalytic loop of protein kinases lies between secondary structure elements αE and β7 and contains an invariant aspartic acid residue (Asp-623 in FLGK) which serves as the catalytic base in the phosphotransfer reaction, abstracting the proton from the hydroxyl group of the substrate tyrosine, serine or threonine. The catalytic loop sequence of FLGK comprises at least residues His-621 to Asn- 628 (amino acid sequence (residues 1666-173 of

SEQ ID NO:1) HRDLAARN), and is identical to that for IRK and most receptor and non-receptor PTKs.

6.1 Production and Purification of FLGK

A recombinant baculovirus (Pharmingen, CA) was engineered to encode the protein of SEQ ID NO:3. Compared to the sequence of human FLGK (SEQ ID NO:1), the protein of SEQ ID NO:3 has a cleavable histidine tag ~~(MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDTSSR)~~ (MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPSSR) fused to the N-terminus to aid in protein purification. Also, three amino acid substitutions were introduced: Cys-488→Ala, Cys-584→Ser and Leu-457→Val. The two cysteine substitutions were made to prevent the formation of disulfide-linked oligomers, which occurs for the native protein. The substitution Leu-457→Val was necessary to introduce a NcoI cloning site near Met-456. The codon for Tyr-766 (TAC) was changed to a stop codon (TAG) and a HindIII-cloning site was generated following this stop codon. These substitutions were introduced into the full length cDNA of human FLGK (SEQ ID NO:4) in m13MP19 by site-directed mutagenesis using an *in vitro* mutagenesis kit according to the manufacturer's protocol (Amersham). The resulting construct was digested with NcoI and HindIII and was ligated into appropriately digested pBlueBac HistagB (Invitrogen). Transfection of insect cells (Sf9) was performed with the BaculoGold transfection system according to the manufacturer's protocol (Pharmingen). Following identification of positive plaques, the recombinant baculovirus was amplified to high titer (5×10^7 virus particles/ml). Sf9 cells were grown in 175-cm^2 flasks to a density of

$2\text{-}3x10^7$ per flask and infected with recombinant baculovirus with a multiplicity of infection (MOI) of ten (10). After 48 hrs., cells were harvested by centrifugation at 3,000g for 35 min. at 4°C and then lysed in lysis buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1.5 mM $MgCl_2$, 1% Triton X-100, 10 μg/ml aprotonin, 10 μg/ml leupeptin and 1 mM phenylmethylsulfonyl fluoride (PMSF)). Lysates were centrifuged in a Sorval RC 5C centrifuge (Dupont) for 1 hr at 4°C at 40,000g followed by ultracentrifugation in an XL-80 ultracentrifuge (Beckman) at 100,000g for 1 hr. After centrifugation, the clarified lysate was passed over a Ni^{2+}-chelating column (Pharmacia), and the bound histidine-tagged fusion protein was eluted with 100 mM imidazole (pH 7.5). Pooled fractions were loaded onto a Mono Q anion exchange column (Pharmacia) and eluted with a NaCl gradient from 0 to 500 mM. The fractions containing the fusion protein were concentrated in a Centricon-30 (Amicon), and the histidine tag was removed by overnight digestion with enterokinase (Biozyme) at 20°C. The digestion was terminated by the addition of aprotonin, leupeptin, PMSF, TPCK (tosyl-L-phenylalanine chloromethyl ketone) and bovine pancreatic trypsin inhibitor (BPTI). The cleaved kinase domain was then separated from the histidine tag on a Superose 12 size-exclusion column (Pharmacia). The eluted kinase domain was further purified on a Mono Q column. The purified kinase domain was analyzed by N-terminal sequencing and mass spectrometry. Five amino acids (SAAGT) (residues 1-5 of SEQ ID NO:2) remained from the histidine tag. The predicted molecular mass was confirmed by mass spectrometry. The amino acid sequence of the purified protein (FLGK) is provided in SEQ ID NO:2.